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Patterning self-assembled monolayers using microcontact printing: a new technology for biosensors?

Milan Mrksich and George M. Whitesides

Self-assembled monolayers (SAMs), formed upon the adsorption of ω-substituted alkanethiols on the surface of gold, allow control of the properties of a surface on the molecular scale. A new technique – microcontact printing (μCP) – can pattern the formation of SAMs, with dimensions on the micron scale. The convenience, low cost, and widespread application offered by SAMs and μCP make this combination of techniques especially suitable for producing and patterning surfaces relevant to biosensors.

A biosensor combines two functions: molecular recognition and signal transduction[1]. The fabrication of a biosensor often requires the properties of surfaces to be tailored and patterned with complex organic functional groups including, for example, ligands for protein recognition, and attachment points for proteins, peptides, carbohydrates and other relevant groups. Convenient, generally applicable methods for producing surfaces are an important part of biosensor technology.

The system of self-assembled monolayers (SAMs) of alkanethiolates on gold is probably the best that is currently available to accomplish the functionalization and patterning of surfaces required by many applications in biomaterials science (for review, see Ref. 2). The convenience and flexibility of SAMs for this purpose has been widely recognized and exploited, especially for homogeneous surfaces. Their stability meets the requirements of most biosensors. In this article, we describe a new technique – microcontact printing (μCP) – that allows SAMs to be patterned[3]. This process can readily generate features down to 1 μm in size, and down to 200 nm with difficulty, and is compatible with complex organic functionality. The process also requires little, or no, access to the photolithographic equipment usually required to generate patterns with these dimensions. The combination of SAMs and μCP provides a remarkably convenient technology for the preparation of patterned surfaces, giving excellent control over surface properties at the molecular level. We believe that this technology will be useful in the production of biosensors.

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Self-assembled monolayers

Alkanethiolates on gold

SAMs of alkanethiolates on gold form when a clean surface of gold is exposed to a solution (or vapor) of a long-chain alkanethiol (RSH, Eqn 1), or dialkyldisulfide (RSSR):

$$RSH + Au(0)_n \rightarrow RS^-Au(I)\cdot Au(0)_n + \tfrac{1}{2}H_2(?) \quad \text{(Eqn 1)}$$

The structure of these SAMs is now well established[4] (Fig. 1). The sulfur atoms coordinate to the gold surface, and the alkyl chains are close-packed, trans-extended and tilted at approximately 30° from the perpendicular to the surface. These monolayers are locally well ordered and have few defects that affect the macroscopic properties of the surface at the 100 nm scale. The terminal functional group of an ω-substituted alkanethiolate dominates the properties of the interface between the SAM and a contacting liquid. The optical characteristics of the system of a SAM supported on gold depend predominantly on the thickness of the underlying gold. SAMs supported on gold 5–10 nm in thickness are transparent, whereas SAMs supported on gold thicker than 100 nm are opaque and reflective[5].

Stability of SAMs

Monolayers of alkanethiolates on gold are stable for a period of several months in air, or in contact with water or ethanol. While some monolayers desorb on heating to temperatures greater than 70°C, others are more stable[6]. In addition, SAMs are stable barriers against corrosion; for example, a SAM of hexadecanethiolate protects the underlying gold from dissolution in highly corrosive etchants, such as aqueous CN^-/O_2 (Ref. 3). Monolayers of alkanethiolates on

gold are sufficiently stable to be useful for many applications in biosurfaces and biomaterials, and have been used for studies of protein adsorption and cell adhesion in aqueous media over periods of several days[7–9].

Mixed SAMs and complex functionality

Adsorption of a mixture of two alkanethiols onto a gold surface allows the production of, so-called, 'mixed' SAMs (Ref. 2). The properties of a mixed SAM can be tailored by varying the ratio of the two alkanethiols in the solution from which they adsorb. The properties of SAMs can be specified further by incorporating complex functional groups (for examples, see Table 1). Alternatively, complex functionality can be introduced after the SAM is formed. This strategy is useful for attaching peptides and proteins to organic surfaces, but is usually less well controlled than methods used to assemble fully preformed components (for examples, see Table 2).

SAMs on other surfaces

Alkylsiloxanes

The second, widely used class of SAMs is siloxanes. These are obtained by the reaction of a hydroxylated surface (usually the native oxide of silicon) with a solution of alkyltrichlorosilane (or triethoxysilane)[18,19]. The reactive trichlorosilane groups condense with hydroxyl groups of the surface, and with neighboring siloxanes. These SAMs have the advantages that they are significantly more thermally stable than alkanethiolates on gold, they do not require evaporation of a layer of metal for preparation of substrates, and they are optically transparent when supported on glass slides. Siloxane monolayers have the disadvantages that they are less ordered than alkanethiolates on gold, and that they are chemically inflexible. The alkyltrichlorosilane groups of the precursors are not compatible with many functional groups, and the variety of surfaces that can be prepared directly (without carrying out reactions on the surface) is limited; the siloxane headgroup hydrolyzes rapidly, even in mild base.

Other organic surfaces

Langmuir–Blodgett (L–B) films were the first system of ordered organic monolayers to be studied. They have been used extensively for the study of biosurfaces, and for applications in the materials sciences[19]. The low stability of L–B films, and the lack of methods for patterning their surfaces, limits their use in the production of biosensors. SAMs obtained by the adsorption of alkylphosphonates on the surface of zirconium oxide[20], and hydroxamic acids on the native oxides of several metals (Ag, Al, Cu, Fe, Ti and Zr) (Ref. 21), are systems that have recently been studied, and that permit control over the properties of organic surfaces. These may be particularly useful when such metal oxides are used.

Interaction of SAMs with biological media

The adsorption of proteins to surfaces is important in many materials and systems used in biotechnology, including biosensors, implants, chromatographic and electrophoretic media, containers for storing and transferring proteins, and containers for cell and tissue culture. As a result, the mechanism of formation of adsorbed protein layers, and their structures, have been studied extensively[22]. Out of necessity, much of this work has used materials that possess structurally ill-defined surfaces, since well-defined, controllable surfaces were not available. The ability to control accurately the nature and density of functional groups

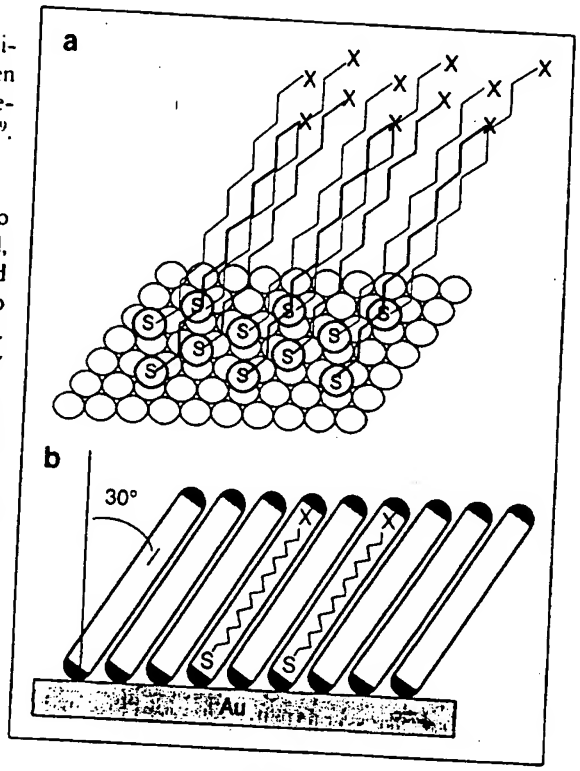

Figure 1

Representation of a self-assembled monolayer (SAM) of alkanethiolates on the surface of gold. **(a)** The sulfur atoms (S) of the alkanethiolates coordinate to the hollow three-fold sites of the gold (1,1,1) surface; the gold atoms (open circles) are arranged in a hexagonal relationship. The alkyl chains are close packed and tilted approximately 30° from the normal to the surface. **(b)** The properties of the SAM are controlled by changing the length of the alkyl chain and the terminal functional group X of the precursor alkanethiol.

Table 1. SAMs containing receptors

Receptor	Ligand	Refs
Porphyrin	O_2 (reduction)	10
Bis(acetoacetate)	Cu(II)	11
Biotin	Streptavidin	12
Resorcin[4]arene	Tetrachloroethylene	13

Table 2. Attachment of proteins to SAMs

SAM FG	Protein (FG)	Refs
$-CO_2H$	Cytochrome *c* ($-NH_2$)	14
$-CO_2H$	Catalase ($-NH_2$)	15
-SSPy	Antibody Fab′ (-SH)	16
$-NH_2$	Polyalanine ($-CO_2H$)	17

Abbreviations: FG, functional group; Py, 2-pyridine.

on the surfaces of SAMs makes them particularly well suited for studies of protein adsorption, and for studies of processes dependent on protein adsorption.

Protein adsorption

The adsorption of several model proteins to SAMs that present different functional groups (e.g. alkyl, perfluoroalkyl, amide, ester, alcohol, nitrile, carboxylic acid, phosphonic acid, boric acid, amine, heterocycle groups) correlates approximately with the hydrophobicity of the surfaces; adsorption on hydrophobic surfaces is often kinetically irreversible, and leads to the formation of a monolayer of protein[7]. Although surfaces presenting charged functional groups have been used to control the adsorption of proteins, the mechanisms for these processes are not well understood; they depend on the molecular composition of the surface, the nature of the protein (e.g. pI, molecular mass, stability, concentration) and the properties of the solution (e.g. pH, ion composition, temperature).

Surfaces that resist the adsorption of proteins

A key requirement in designing surfaces that interact specifically with designated proteins is preventing unwanted adsorption of other proteins. Hydrophilic and flexible polymers [especially polyethylene glycol (PEG) and polysaccharides] have traditionally been used to passivate surfaces against protein adsorption[23]. SAMs presenting short oligomers of ethylene glycol (EG), which are prepared using the alkanethiols $HS(CH_2)_{11}(OCH_2CH_2)_nOH$ $(EG)_n$, (n = 2–7), resist the kinetically irreversible adsorption of proteins to their surfaces[8]. The effectiveness of these surfaces is highlighted by the observation that they even resist the adsorption of 'sticky' proteins, such as fibrinogen. The degree to which a SAM resists the adsorption of proteins can be controlled, either by varying the length of the $(EG)_n$ unit, or by adjusting the ratio of components in a mixed SAM comprising methyl- and $(EG)_n$-terminated alkanethiolates. The $(EG)_n$-terminated SAMs may represent a general class of surfaces onto which ligands, peptides or proteins can be specifically immobilized.

Attachment and growth of cells on SAMs

The attachment of anchorage-dependent cells to the extracellular matrix (ECM) – the network of polysaccharides and proteins (i.e. fibronectin, laminin, vitronectin, heparin, collagens) that makes up a substantial part of most tissue – is mediated by specific interactions between the integrin receptors of the cellular membranes and short peptide sequences of the ECM. A common strategy for controlling the attachment of cells onto a surface relies on specifying the adsorption of ECM proteins onto the surface. The attachment of rat basophilic leukemia cells to SAMs presenting a range of functional groups [methyl, trifluoromethyl, alcohol, carboxylic acid, dimethylamino, $(EG)_n$] has been studied; the cells attached to surfaces that promoted adsorption of laminin[9]. Massia and Hubbell demonstrated that siloxane SAMs presenting the peptides Arg-Gly-Asp or Tyr-Ile-Gly-Ser-Arg (the recognition sequences for fibronectin and laminin, respectively), supported the adhesion and spreading of fibroblast cells without the need for coating the surfaces with ECM proteins[24]. These early examples suggest that the attachment of biomolecules to SAMs will make possible the design of surfaces with sophisticated control over the functions of attached cells.

Methods for patterning surfaces

Microcontact printing

Several simple techniques for patterning SAMs of alkanethiolates on gold have been developed – microwriting[25], micromachining[26] and μCP (Ref. 27). The most useful of these techniques is μCP: it can routinely form features of sizes ranging down to 1 μm, and features as small as 200 nm have been formed using this technique (Fig. 2). The process of μCP starts with an appropriate relief structure, from which an elastomeric stamp is cast. This 'master' template is usually generated photolithographically, but can be produced using other procedures (for example, using commercially available diffraction gratings[3,27]). The stamp (usually made from polydimethylsiloxane) is 'inked' with a solution of alkanethiol in ethanol, dried and brought into contact with a surface of gold. The alkanethiol is transferred to the surface only at those regions where the stamp contacts the surface. This process produces a pattern of SAM that is defined by the pattern of the stamp. It is possible to pattern areas with sizes of several cm^2, and with edge resolution of features better then 50 nm, due to conformal contact between the elastomeric stamp and the surface, the rapid reaction of thiols with gold and the autophobicity of the alkanethiol ink. Multiple stamps can be produced from a single master, and each stamp can be used hundreds of times without any loss of quality of the printed patterns. Because μCP is a technique that relies on molecular self-assembly and does not require stringent control over the laboratory environment, it can produce patterns at low cost, relative to methods that use photolithography.

Photolithography

Methods for patterning siloxane monolayers at the micron scale have relied exclusively on photolithography[28]. In the 'lift-off' technique, a silicon substrate

coated with a photoresist is irradiated with ultraviolet (UV) light through a mask containing the pattern to be reproduced. The irradiated regions of photoresist are removed selectively, and a SAM is formed on the exposed regions of silicon oxide by immersing the substrate in a solution of alkyltrichlorosilane, or a hydrolyzed oligomer from the alkylsilane. The remaining photoresist is then removed, and these regions are derivatized with a SAM containing a different terminal functional group. This method can routinely generate patterns with features down to approximately 300 nm in size, but is limited in the range of functional groups that can be patterned. Irradiation with UV light of a SAM of alkanethiolates on gold oxidizes the thiolates to sulfonates. The alkylsulfonates can then be replaced with another alkanethiol[29]. Photolithographic patterning of SAMs has been demonstrated using this methodology. However, patterns formed by UV lithography showed less resolution than patterns formed by μCP (Refs 7,30).

Microcontact printing followed by selective etching generates microstructures of gold and of silicon

The ability of a SAM of hexadecanethiolate to protect the underlying gold from dissolving in an aqueous cyanide etch forms the basis for a simple technique to make a range of structures with well-defined patterns and morphologies[31]. Exposure of a patterned SAM (usually prepared using μCP) to the selective etchant results in the dissolution of gold at those regions not protected by a SAM (Fig. 2). This method can be used, for example, to produce arrays of microelectrodes[2]. The microstructures of gold are also useful as masks, which protect the underlying silicon from an etch. Exposure of a silicon substrate, patterned with features of gold, to an anisotropic alkaline etch results in the controlled dissolution of silicon, thereby generating features of silicon with defined geometries. The shape of the three-dimensional features is determined by the etching conditions (Fig. 3).

Applications

Patterned adsorption of proteins on surfaces

Many applications require control over the spatial distribution of proteins or other biomolecules adsorbed on surfaces. The μCP technique has been used to pattern a SAM into regions terminated with methyl- and $(EG)_6$ groups with dimensions down to 1 μm. Exposure of the patterned substrate to a protein-containing solution resulted in the irreversible adsorption of protein to the hydrophobic regions of the SAM. Scanning electron microscopy (SEM) was used to visualize the pattern of the protein adlayer[7,32] (Fig. 4). Alternatively, proteins have been immobilized onto a surface containing patterned regions of a reactive functional group, provided that the complementary regions are resistant to the adsorption of proteins. Bhatia *et al.* have described the patterning of siloxane film terminated in thiol groups by irradiation with UV light through a mask[33]. The fluorescent protein, phycoerythrin, was immobilized to thiol groups in regions that were protected from the UV light by the mask. Photo-induced oxidation of the thiol groups in regions of the surface that were irradiated presumably produced negatively charged sulfonate groups, which resisted the adsorption of the protein[33]. Immobilized arrays of hundreds of different peptides and nucleic acids were created by combining solid-phase organic synthesis with photolithographic techniques. Furthermore, Fodor and co-workers prepared a library comprising 1024 different peptides and assayed the binding of each member to a fluorescently labeled antibody in a single experiment[34].

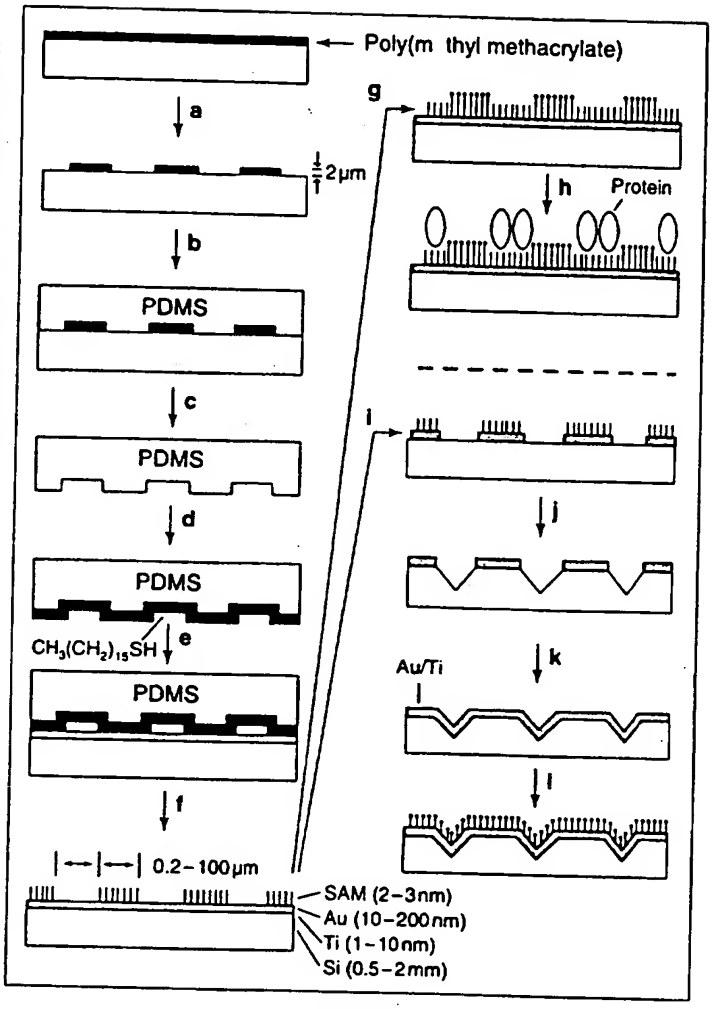

Figure 2

Procedure for patterning self-assembled monolayers (SAMs) using microcontact printing. Photolithography or other methods generates a mask containing features of the pattern to be reproduced (**a**). A polydimethylsiloxane (PDMS) prepolymer is poured onto the master pattern, allowed to cure (**b**), and peeled away from the master (**c**). The stamp is inked with alkanethiol (**d**) and used to transfer the alkanethiol to the surface (**e**); this transfer (**f**) forms a patterned SAM (the representation of the SAM implies no structure). Exposing the gold substrate to a solution of a different alkanethiol derivatizes the bare regions (**g**); immersion of the patterned SAM in a protein-containing solution results in adsorption of protein preferentially on one type of surface of the SAM (**h**). Bare regions of gold remaining after the initial printing (**f**) can also be removed selectively by etching (**i**). Anisotropic etching of the silicon exposed by removing the gold generates defined surface topographies (**j**). After cleaning the substrate, a layer of gold can be deposited (**k**); the properties of this contoured surface can be controlled by forming a SAM of alkanethiolates (**l**).

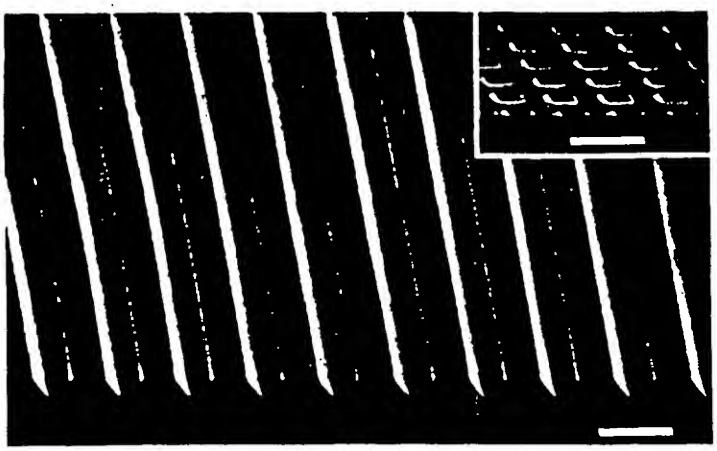

Figure 3

Features of silicon generated by microcontact printing (μCP) followed by selective etching. Fracture profile of a surface with parallel trenches 3 μm in width. (Scale bar represents 5 μm.) The inset shows a pattern of square pyramidal pits generated by etching a surface that was stamped twice. (Scale bar represents 10 μm.)

Patterned cell attachment

Methods for patterning the adsorption of ECM proteins to surfaces form the basis for patterning the attachment of cells to surfaces[35,36]. Kleinfeld *et al.* prepared siloxane SAMs, containing regions terminated in methyl and amino groups[36]. Cerebellar cells plated in media containing serum attached and grew only on the ionic, rather than the hydrophobic, regions of the surface, whereas cells plated in the absence of serum attached to all regions of the surface. Similar findings were obtained using SAMs of alkanethiolates on gold[23]. Presumably, there is a kinetic preference for serum proteins that do not promote attachment of cells to adsorb on the hydrophobic regions.

SAMs patterned into ~20 × 50 μm islands permit the control of the attachment of individual cells[37]. Using μCP of hexadecanethiol a surface with hydrophobic islands of defined shape and size that were separated by regions of $(EG)_6$-terminated SAM was created. Exposure of this substrate to a solution of laminin resulted in adsorption of protein on the hydrophobic regions. When hepatocytes were plated on this substrate, they attached to the rectangular islands and conformed to the shape of the underlying pattern (Fig. 5). The size of the islands controlled the DNA synthesis, cell growth and protein secretion of the attached cells. The ability to pattern defined arrays of immobilized cells makes the construction of new types of whole-cell-based sensors possible.

Contoured surfaces

Several groups have used photolithography to make surfaces contoured into grooves and ridges; these features strongly affect the behavior and growth of attached cells[38,39]. Surfaces with arrays of grooves of varying dimensions controlled the alignment and orientation of attached mammalian cells[38], whereas surfaces with arrays of ridges directed the motility and induced the differentiation of the fungus *Uromyces*[39].

SAMS as components of analytical devices

A number of analytical techniques that are useful in biotechnology and biochemistry measure the properties of interfaces: for example, the change in mass at a surface (surface acoustic wave, quartz crystal microbalance and acoustic plate mode sensors)[40], or the change in the index of refraction near a surface [surface plasmon resonance (SPR) (Ref. 41), interferometric waveguide[42], ellipsometry and total internal reflectance fluorescence]. Proteins, antibodies and

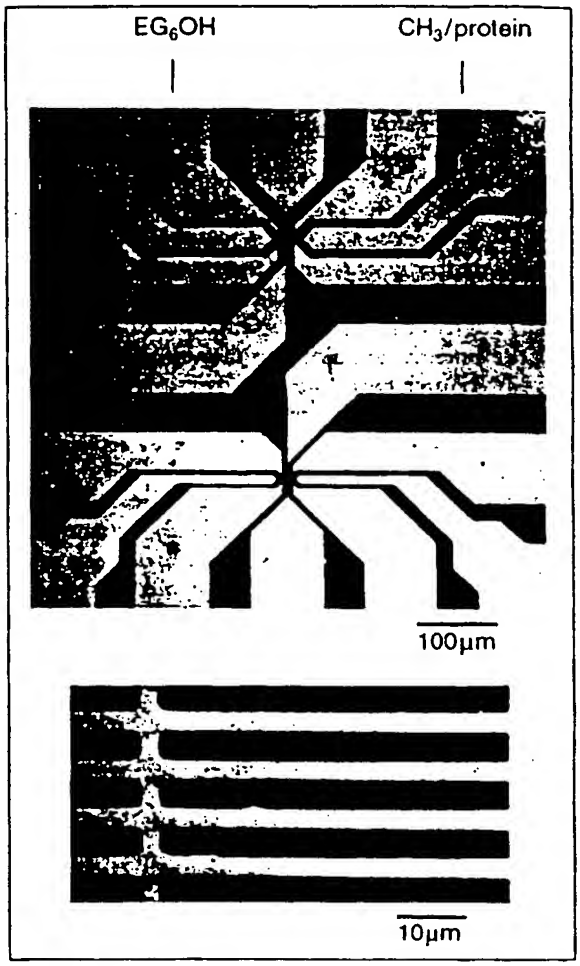

Figure 4

Scanning electron microscope (SEM) micrographs of fibrinogen adsorbed on a patterned SAM. A patterned hexadecanethiolate self-assembled monolayer (SAM) on gold was formed by μCP, and the remainder of the surface was derivatized by exposure to a hexa(ethylene glycol)-terminated alkanethiol $[HS(CH_2)_{11}(OCH_2CH_2)_6OH]$. The patterned substrate was immersed in a solution of fibrinogen (1 mg ml^{-1}) in PBS buffer for two hours, removed from solution, rinsed with water, and dried. Fibrinogen adsorbed only to the methyl-terminated regions of the SAM, as illustrated by the dark regions in the SEM micrograph. Secondary electron emission from the underlying gold is attenuated by the protein adlayer. The top image shows a pattern of the type used in microelectronics circuits. The bottom image demonstrates that microcontact printing is useful for patterning the adsorption of proteins at dimensions on the micron scale.

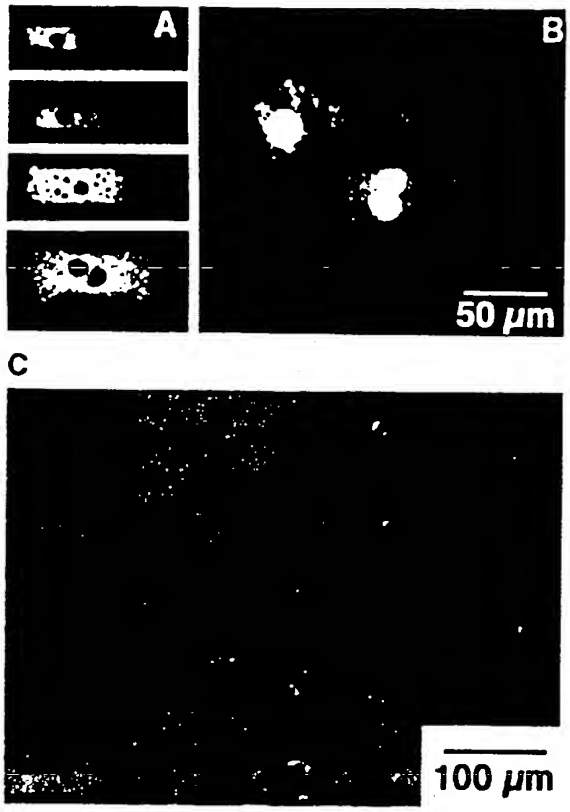

Figure 5

Control of the shape and size of hepatocytes on patterned self-assembled monolayers (SAMs) comprising methyl- and (ethylene glycol)-terminated regions. Exposure of the SAM to a solution of laminin resulted in selective adsorption of the protein on the hydrophobic regions; cells attached preferentially to the protein-coated islands. **(A)** The hepatocytes conformed to the shape of the features of the patterned SAM. **(B)** Cells adherent to an unpatterned SAM were characterized by spreading and cell–cell contacts. **(A)** and **(B)** are fluorescent micrographs of cells that were stained with 5-bromodeoxyuridine (BrdU). Only cells on non-patterned substrata showed nuclear uptake of BrdU (DNA synthesis). Cells adherent to the rectangular islands were prevented from entering S phase and undergoing chromatid duplication. **(C)** Low-magnification view of SEM of cells adherent to islands of different size. Reproduced, with permission, from Ref. 37.

nucleic acids have been conjugated to the surfaces of these devices to make bio-specific sensors. An important, general problem in most of these sensors is the non-specific adsorption of proteins. A common strategy for minimizing this has been to coat the surface with a protein, usually bovine albumin, that resists further adsorption of proteins. We believe that SAMs comprising $(EG)_n$ groups, or other functional groups that resist the adsorption of proteins, will be more effective, and provide better control, at preventing unwanted adsorption of proteins to the surfaces of biosensors.

Electrochemistry and microelectrodes

SAMs can be used to modify the properties of electrodes by insulating the surface, and by providing redox-active groups. A SAM of hexadecanethiolate blocks the transfer of electrons between a gold electrode and an aqueous solution of a redox-active molecule; a SAM terminated in an electroactive group (e.g. ferrocene or quinone) mediates the transfer of electrons to the underlying gold electrode. Incorporation of an electroactive group that has an electrochemical potential sensitive to the concentration of protons into a SAM is the basis for a pH indicator[43]; this principle can be extended to other analytes. A rapid assay for the analysis of biological analytes, based on electrochemiluminescence from tris-bipyridine ruthenium(II) tags, has been developed[44,45]. This type of technology is well suited for SAMs. Using μCP, regions of SAM terminated in electroactive groups (or regions of bare gold) can be patterned with dimensions in the micron range. These microelectrodes have several advantages over traditional electrodes, including small currents, fast response times, applicability to small sample volumes (even single mammalian cells) and utility in media of low conductivity[46].

Optically addressable SAMs

Analytical methods that probe the properties of interfaces using optical phenomena have the advantages that they are fast, non-invasive and inexpensive. A diffraction-based humidity sensor has been made by preparing a surface having hydrophobic and hydrophilic regions with micron-scale periodicity[47]. Condensation of water on the hydrophilic regions results in a regular pattern of condensation figures that acts as a diffraction grating: the intensity of a diffracted beam is a quantitative measure of the local humidity.

A difference interferometer has been constucted by modifying the surface of a TiO_2–SiO_2 waveguide with a siloxane monolayer to which the antibody against hepatitis B antigen was conjugated. This sensor could be used to measure the binding of hepatitis B down to a concentration of 2×10^{-13} M in undiluted serum[42]. An optical technique based on SPR measures the resonance angle of light reflected from a glass slide coated with a layer of gold. We prepared a SAM presenting a nickel(II) complex and used SPR to measure the binding of a protein having several histidine residues at its C-terminus (Fig. 6) (G. B. Sigal, C. Bamdad, A. Barberis, J. Strominger and G. M. Whitesides, unpublished).

Implications for fabrication of biosensors

SAMs, especially those formed from the adsorption of alkanethiols on gold, are the best system now available to accomplish the functionalization of surfaces with complex, reactive or unstable organic groups of the sorts most relevant in bioanalytical chemistry. The new capabilities provided by SAMs make possible the fabrication of new types of devices. Other features that make SAMs of alkanethiolates on gold attractive for use in the fabrication of biosensors are: the optical transparency of these films (when supported on gold with thickness <100 Å) (Ref. 5); the electrical conductivity of the gold; and the stability of these monolayers. The ability to pattern the formation of SAMs

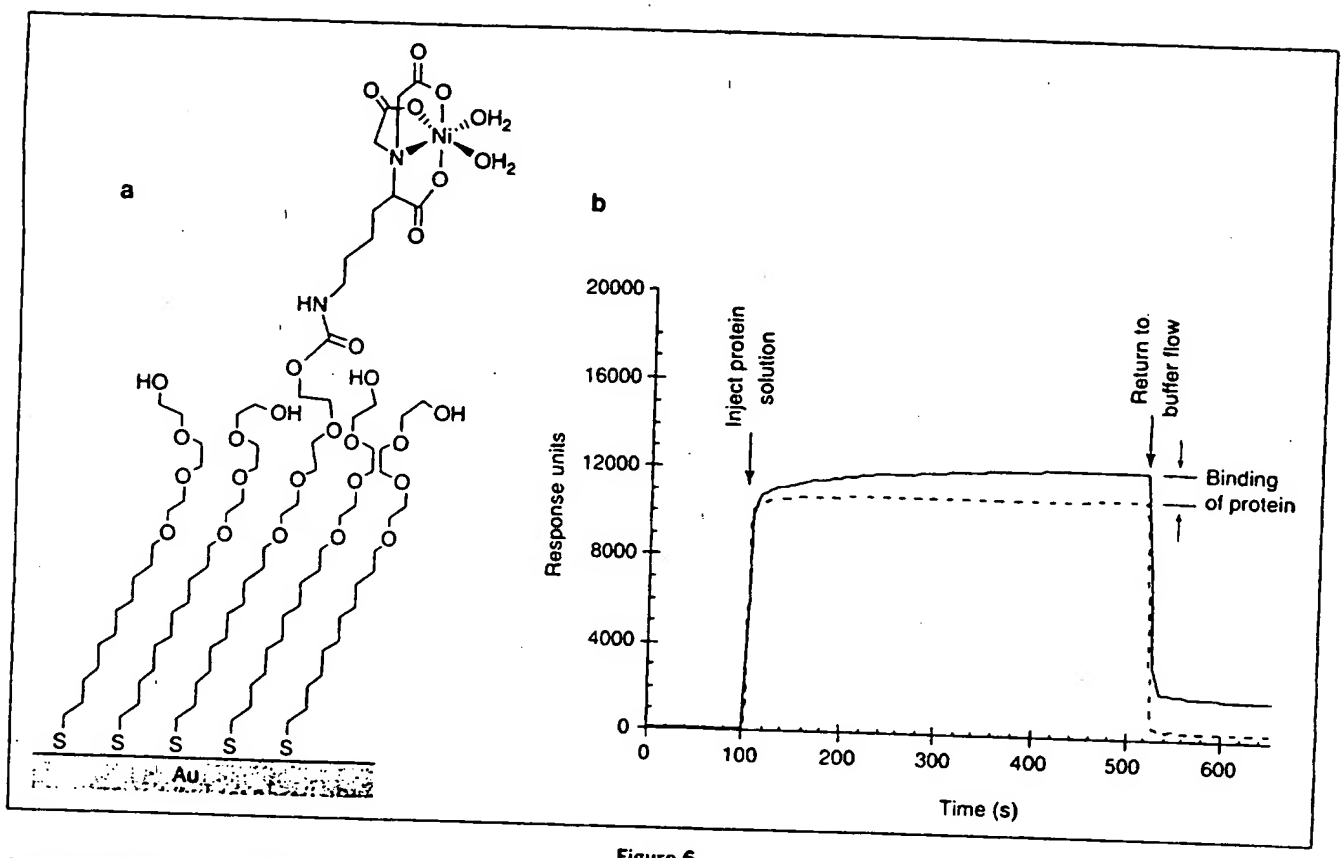

Figure 6

Surface plasmon resonance (SPR) was used to measure the rate and quantity of binding of a His-tagged T-cell receptor construct to a self-assembled monolayer (SAM) terminated in ethylene glycol $(EG)_3$ groups and Ni(II) complexes. **(a)** The mixed SAM contains ~5% of the Ni(II)-functionalized alkanethiol. Imidazole rings of the His-tagged protein replace the water ligands of the Ni(II) complex. **(b)** The resonance angle of light reflected from the SAM/gold was plotted in arbitrary units against time. A large response, due to the change in index of refraction of the solution, was observed upon introduction of protein into the flow cell (dashed curve). The difference between the measured response and this background signal represents binding of the His-tagged protein to the SAM.

in simple ways using μCP allows the fabrication of multi-array biosensors; for example, those that use optical diffraction or electrochemiluminescence. The attributes of SAMs and μCP described in this review are just beginning to find applications in sensors and biomaterials. Many more applications will certainly follow.

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book reviews

Successfully navigating the regulatory maze

Regulatory Practice for Biopharmaceutical Production

edited by A. S. Lubiniecki and S. A. Vargo, Wiley–Liss, 1994. UK£76.95 (ix + 555 pages) ISBN 0 471 04900 X

This book is an excellent and authoritative compendium of regulatory practice as applied to biopharmaceuticals. Co-edited by representatives of the biopharmaceutical industry and the US Food and Drug Administration (FDA), this balanced theme is continued throughout, with individual chapters contributed by senior notables from biotechnology/pharmaceutical companies and the Center for Biologics Evaluation and Research. Although written primarily from a US viewpoint, the approach is common for requirements worldwide, reflecting the move towards harmonization between the international regulatory bodies, and also the multinational status of the industry. In addition, there are specific chapters dealing with the approval process in the European Union and regulations in Japan.

The book's content is mainly confined to discussion of the manufacture of specific gene products in heterologous hosts – which the editors encapsulate as 'novel biotechnology' – with reference to therapeutic, *in vivo* diagnostic and prophylactic products licenced and in clinical trials. The early chapters comprise detailed reviews of the general issues relating to the manufacturing, testing and regulation of biopharmaceuticals. There are overviews of the licensing and approval process in the USA, together with an informed discussion on risk assessment, government policy and the FDA's philosophy on regulation. These are followed by more detailed reviews of the key elements for manufacture and approval, including determination of the genetic stability of the host cell and product, continuous cell lines and contaminant testing, characterization of recombinant polypeptides, quality control, process design and formulation.

The middle chapters use specific products to highlight the regulatory issues that must be addressed. These are well considered and evidently written from personal experience, either from a manufacturing or regulatory viewpoint. Examples are wide ranging and include cytokines, growth factors, peptide hormones, coagulation factors, erythropoietin, monoclonal antibodies and hepatitis B surface antigen.

The later chapters deal with facility and equipment design, licensing and the highly topical issue of computer system validation. There is a chapter devoted to water systems for biotechnology facilities, a crucial utility when dealing with parenteral products, and potentially one of the major heartaches when undergoing regulatory inspections. There are also useful reviews on microheterogeneity of biological products and regulatory aspects of contract manufacturing. This latter topic is highly relevant in the 1990s, as companies seek to constrain capital costs prior to completion of clinical trials and proven efficacy of their products. Contracting-out, and the use of multi-product facilities, is essential for the small and medium-sized biotechnology companies, given the range of products now in development.

The final chapter entitled 'Unresolved issues' recognizes the continuing debate on how best to handle 'novel biotechnology'. It covers such issues as cellular DNA, retroviral contaminants, human